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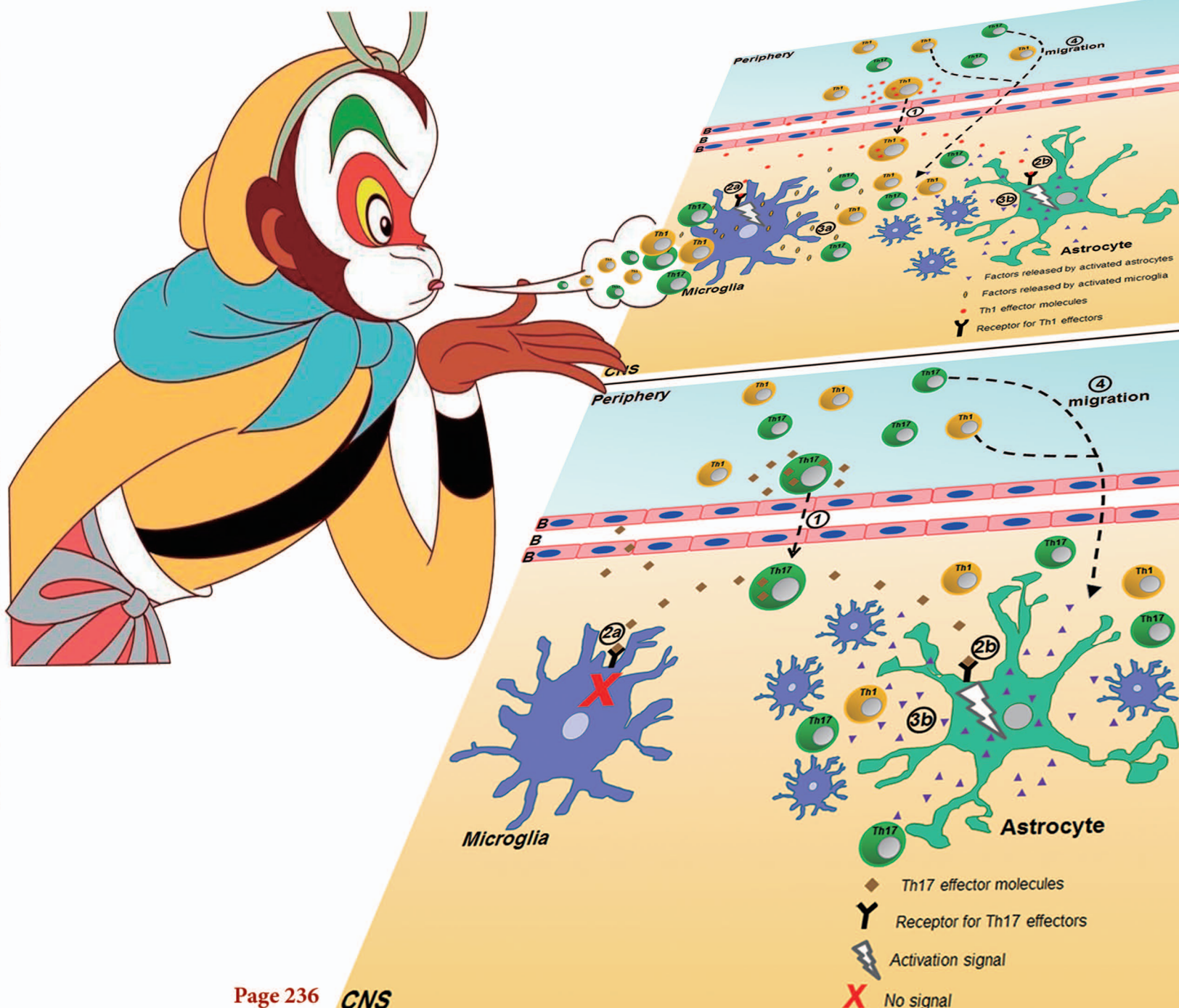
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Neural Regeneration Research



● PERSPECTIVE

Retinal ganglion cell neuroprotection by growth factors and exosomes: lessons from mesenchymal stem cells

Retinal ganglions cells (RGCs) are responsible for propagating electrochemical information from the eye to the brain along their axons which make up the optic nerve. The loss of RGCs is characteristic in several conditions such as glaucoma and traumatic optic neuropathy and leads to visual loss and blindness. While no therapy exists to directly treat RGCs, the use of bone marrow-derived mesenchymal stem cells (BMSCs) has shown promise in eliciting significant RGC neuroprotection. Their efficacy is proven in both *in vitro* (retinal co-culture (Mead et al., 2014) and organotypic retinal explants (Johnson et al., 2014)) and *in vivo* (ocular hypertension (Johnson et al., 2010) and optic nerve crush (Mesentier-Louro et al., 2014)) models from multiple laboratories and are currently being investigated in clinical trials (reviewed in Mead et al., 2015). While other MSCs exist, such as those isolated from adipose tissue, umbilical cord blood and dental pulp, and have even been demonstrated to act differently in neuroprotective assays (Mead et al., 2014), BMSCs are the most widely studied and the predominant MSC undergoing clinical trials. Although BMSCs do not replace retinal cells and their mechanism of action is exclusively through the secretion of neuroprotective compounds, BMSCs represent an exciting candidate for cellular therapy of the retina. A large body of evidence exists for the efficacious use of BMSCs in several eye disease models and over ten stage 1 clinical trials are underway (Reviewed in Mead et al., 2015). While many of these trials have now reported good findings with successful transplantation into patients, the safety aspect of delivering living, dividing cells into the eye can still be questioned given the recent case study of three patients going blind after receiving intravitreal adipose-derived MSCs (Kuriyan et al., 2017). Issues such as hemorrhage and retinal detachment were observed and may reflect a possible side effect of intravitreal cell therapy. What is also not clear is the “shelf-life” of the BMSCs, particularly considering that they will need to be stored in liquid nitrogen, or grown and maintained at 37°C/5% CO₂. These requirements add further costs and expertise needed for such a treatment while also introducing variability, particularly since the longer the cells are grown the lower the titers of secreted neuroprotective compounds (Mead et al., 2014). Another issue is that the BMSCs secretome contains a wide variety of compounds, some of which, such as vascular endothelial growth factor (VEGF), may be detrimental to the retina in high concentrations. While using BMSCs as a therapy is one avenue of research, understanding of their mechanism and the development of new treatments, independent of the cells themselves is equally important and would circumnavigate much of the issues detailed above. Our research has identified two very different modalities by which BMSCs protect RGCs, secretion of multiple neuroprotective peptides, of which platelet-derived growth factor (PDGF)-AA was the most neuroprotective, and secretion of extracellular vesicles including exosomes.

PDGF-AA: BMSCs are known to secrete a variety of neurotrophic factors including nerve growth factor and brain-derived neurotrophic factor which through the activation of Tropomyosin receptor kinase A (TrkA) and Tropomyosin receptor kinase B (TrkB), elicit neuroprotection. In an effort to identify additional factors with neuroprotective efficacy, BMSC’s secretome was analyzed and PDGF-AA was found to be enriched in comparison to

the secretome of fibroblasts, which unlike BMSCs lacked neuroprotective efficacy. PDGF-AA is a homodimer that interacts with PDGF receptor- α (PDGFR α). Blockade of PDGFR α significantly reduced BMSCs neuroprotection *in vitro* whereas intravitreal delivery of recombinant PDGF-AA promoted significant neuroprotection of RGCs in an ocular hypertensive rat model (Johnson et al., 2014). A second study demonstrated in the same model that an intravitreal injection of PDGF-AA preserved RGCs synaptic density within the inner plexiform layer (Chong et al., 2016).

While we initially hypothesized that PDGF-AA would act directly on RGCs to elicit its neuroprotective effect, the mechanism appeared more complicated given that RGCs do not express PDGFR α . In a recent study to elucidate the retinal targets of PDGF-AA, EGFP was expressed under the *PDGFR α* promoter (Takahama et al., 2017). PDGFR α expression was localized to astrocytes within the ganglion cell layer and Type 45 GABAergic wide-field amacrine cells in the inner nuclear layer. While the mechanism of action is yet to be confirmed, RNAseq of PDGFR α ⁺ amacrine cells and astrocytes is planned to determine changes following PDGF-AA stimulation. We hypothesize that astrocytes and/or amacrine cells, in response to PDGFR α activation, secrete factors neuroprotective for injured RGCs.

Exosomes: Exosomes are small extracellular vesicles ranging in size between 40–100 nm. Typically in the literature they are often isolated through ultra-centrifugation and thus also include microvesicles, which are extracellular vesicles ranging in size between 100–1,000 nm. While microvesicles are released from cells *via* outward budding of the plasma membrane, exosomes are formed within multivesicular bodies which upon its fusion with the plasma membrane, are secreted into the extracellular space. Although we use the term “exosome”, “small extracellular vesicle” is also an appropriate term, since some smaller microvesicles can be present in the preparation.

Exosomes contain proteins, lipids, mRNAs and microRNAs (miRNAs) which can be transported and delivered to other cells. Recipient cells can translate these mRNAs into proteins as well as have gene expression regulated by the miRNAs. Unlike peptides such as PDGF-AA, because exosomes deliver their cargo directly into cells, they are not dependent on the expression of specific receptors. We recently demonstrated that exosomes derived from BMSCs are able to protect RGCs from death in rat models of optic nerve crush (ONC) (Mead and Tomarev, 2017) and glaucoma (Mead et al., 2018).

Exosomes were found to integrate indiscriminately into the ganglion cell layer and the neuroprotective effect was dependent on efficient delivery of miRNAs. Thus, unlike PDGF-AA that acts on the PDGFR α of astrocytes/amacrine cells, exosomes function through several mechanisms including the direct modulation of mRNAs translation through miRNA-mediated knockdown. Treatment of purified cultures of RGCs with exosomes, and subsequent neuroprotection demonstrates that the mechanism is, at least partially, mediated through RGCs directly (Mead et al., 2018). While few studies exist that have tested exosomes as a neuroprotective therapy, a similar observation was seen on cultured cortical cells, suggesting the effect is not specific to RGCs and thus, exosomes may benefit other injured central nervous system (CNS) neurons (Zhang et al., 2017). Interestingly, this group also attributed the effects of BMSCs exosomes to their miRNA cargo.

Our recent study highlighted over 40 miRNAs overabundant in BMSCs exosomes in comparison to fibroblast exosomes (Mead et al., 2018). The exact miRNA or combination of miRNAs responsible for the exosome-mediated neuroprotection is the subject of our current investigations.

Future work: BMSCs have shown great promise in several mod-

els of retinal injury and by several groups. Since the mechanism of action appears to be multifactorial, a great amount can be learnt by studying their secretome to determine new novel neuroprotective pathways.

Future studies are to focus on the mRNA changes of RGCs (in the case of exosome treatment) and astrocytes/amacrine cells (in the case of PDGF-AA) treatment. By understanding the mRNA changes in these cell types following their respective treatments, the mechanism of action can be better understood. In the case of exosomes, cross referencing miRNAs abundantly present in BMSCs with mRNAs downregulated in RGCs after exosome treatment will further help to narrow down the signalling pathways responsible for the neuroprotective effect. By determining the miRNAs both already abundant in healthy RGCs or downregulated after ocular hypertension/optic nerve injury, candidate exosome-derived miRNAs can be further narrowed down.

By understanding the mechanism of action of each MSC-derived neuroprotective compound, combinatorial therapies can be developed that target multiple pathways. For example, while it appears that PDGF-AA and exosomes work through different mechanisms, the downstream neuroprotective signalling pathways may be shared. One should also keep in mind that some differences may exist in the neuroprotective mechanisms activated in the human and rodent retinas. For example, a recent paper demonstrated that PDGF-AB but not PDGF-AA was neuroprotective in human retinal explant cultures. It was also demonstrated that blockade of PDGF signalling, unlike in rodent retina, did not reduce MSC-induced neuroprotection, suggesting greater redundancy in the mechanism of action on human RGCs (Osborne et al., 2018). Therefore, neuroprotective compounds identified in rodent models should be tested in larger animal models of glaucoma and traumatic optic neuropathy before testing in humans.

After intravitreal transplantation, BMSCs remain in the eye and presumably continue to secrete neuroprotective compounds on the injured retina. In contrast, administration of purified neuroprotective compounds (exosomes or PDGF-AA) is short lived with the effect ranging from several days to a month. In our previous study utilizing a rat model of glaucoma characterized by 2 months of ocular hypertension, exosomes elicited neuroprotection if injected monthly but not if injected only once at the beginning (Mead et al., 2018). In contrast, PDGF-AB treatment of human retinal wholemounts are more short lived with the peptide decreasing in concentration by 80% five days after treatment (Osborne et al., 2018). Thus, for a cell free therapy to be effective, the longevity must be addressed to avoid patients needing repeat injections.

Another important consideration is that the RGCs are not a homogenous population but rather, a collective of about 50 subtypes in mice. While injury is known to differentially affect RGC subtypes, it is equally expected that treatment will target specific subtypes. The combinatorial nature of MSC's secretome may target multiple RGC subtypes while isolating specific neuroprotective compounds may leave certain subtypes unprotected.

Conclusion: BMSCs are known to secrete many compounds that act therapeutically on many organs and diseases. While a treatment in themselves, BMSCs offer an important research tool to study their secretome and discover new novel signalling pathways that be exploited for clinical effect.

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Open peer review reports:

Reviewer 1: Melissa Renee Andrews, University of St Andrews, UK.

Comments to authors: The Perspective article is a timely article which describes work using BMSCs in retinal injury models. The work specifically describes potential mechanisms by which BMSCs promote neuroprotection in retinal ganglion cells. Although the field is relatively small in terms of use of BMSCs in retinal injury, the authors and collaborators have done significant and high quality work in this field.

Reviewer 2: Steven Levy, MD Stem Cells, USA.

Reviewer 3: James G. Patton, Vanderbilt University, USA.

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